Buckley, K. E.; Fisher, L. J.; MacKay, V. G. "Electron Capture Gas Chromatographic Determination of Traces of Formaldehyde in Milk as the 2,4-Dinitrophenylhydrazone". *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 655–657.

Florence, E.; Milner, D. F. "Determination of Free and Loosely Protein-bound Formaldehyde in the Tissues of Pigs Fed Formalin-treated Skin Milk as a Protein Supplement". *J. Sci. Food Agric.* **1981**, *32*, 288–292.

Galli, C. L.; Ragusa, C.; Resmini, P.; Marinovich, M. "Toxicological Evaluation in Rats and Mice of the Ingestion of a Cheese made from Milk with Added Formaldehyde". *Food Chem. Toxicol.* **1983**, *21*, 313–318.

Hayashi, T.; Reece, C. A.; Shibamoto, T. "Gas Chromatographic Determination of Formaldehyde in Coffee via Thiazolidine Derivative". *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 101–105.

Heck, H. d'A.; White, E. L.; Casanova-Schmitz, M. *Determination* of Formaldehyde in Biological Tissues by Gas Chromatography/Mass Spectrometry". *Biomed. Mass Spectrom.* **1982**, *9*, 347–353.

Heck, H. d'A.; Casanova-Schmitz, M.; Dodd, P. B.; Schachter, E. N.; Witek, T. J.; Tosun, T. "Formaldehyde (CH_2O) Concentrations in the Blood of Humans and Fischer-344 Rats Exposed to CH_2O Under Controlled Conditions". *Am. Ind. Hyg. Assoc. J.* **1985**, *46*, 1–3.

Kreula, M.; Rauramaa, A. "Transfer of Formaldehyde from Feed to Milk during the Feeding of Fresh Cut Grass Treated with Formaldehyde-containing Preservative". *J. Sci. Agric. Soc. Finland* **1976**, *48*, 154–157.

Lawrence, J. F.; Iyenger, J. R. "The Determination of Formaldehyde in Beer and Soft Drinks by HPLC of the 2,4-Dinitrophenylhydrazone Derivative". *Int. J. Environ. Anal. Chem.* **1983**, *15*, 47–52.

Little, T. M.; Hills, F. J. "Sub-plots as Repeated Observations". In *Statistical Methods in Agricultural Research*; University of California: Davis, CA, 1972.

Mills, S. C.; Sharry, L. F.; Cook, L. J.; Scott, T. W. "Metabolism of [^{14}C]Formaldehyde when Fed to Ruminants as an Aldehyde-Casein-Oil Complex". *Aust. J. Biol. Sci.* **1972**, *25*, 807–816.

Modler, H. W.; Muller, P. G.; Elliot, J. T.; Emmons, D. B. "Economic and Technical Aspects of Feeding Whey to Livestock". *J. Dairy Sci.* **1980**, *63*, 838–855.

Syrjälä-qvist, L.; Setälä, J. "Formaldehyde Content of Milk. 1. Cows Fed on Protein Concentrates Treated with Different Amounts of Formaldehyde". *J. Sci. Agric. Soc. Finland* **1982a**, *54*, 63–67.

Syrjälä-qvist, L.; Setälä, J. "Formaldehyde Content of Milk. 2. Cows Fed on Grass Silage Preserved With Formaldehyde-containing Additive and on Formaldehyde-treated Urea. *J. Sci. Agric. Soc. Finland* **1982b**, *54*, 69–76.

Tome, D.; Naulet, N.; Kozlowski, A. "^{13}C-N.M.R. Characterization of Formaldehyde Bonds in Model Mixtures and Proteins Containing Lysine. *Int. J. Peptide Protein Res.* **1985**, *25*, 258–266.

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Isolation and Characterization of Low Molecular Weight Protein from Mustard (*Brassica juncea*)

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The low molecular weight protein fraction from *Brassica juncea*, the mustard seed, has been purified to homogeneity by ammonium sulfate fractionation followed by gel filtration on a Sephadex G-75 column. The protein contains 0.2% carbohydrate, is free from phosphorus, and is rich in its available lysine content (6.7 ± 0.4%). The protein has a sedimentation coefficient of 1.7 S and molecular weight of 22 900 ± 2000 and consists of two polypeptide chains of molecular weights 13 000 and 12 000. The protein has a low content of aromatic amino acids and is free from chromogenic impurities like phenolic acids and nucleic acids; it has an absorption maximum at 278 nm and a shoulder at 288 nm. The fluorescence emission maximum is at 335 nm. The optical rotatory dispersion spectrum indicates a trough at 232 nm with −6000° cm^2 dmol^{-1} of molecular rotation. The near-ultraviolet and far-ultraviolet circular dichroism measurements indicate that the protein differs from the high molecular weight protein fraction in terms of its tertiary and secondary structure.

Mustard (*Brassica juncea*) is one of the major oilseeds of India. The defatted meal is of nutritionally superior quality and can be incorporated in human and animal food and feed after the removal of glucosinolates (Ohlson, 1985). An understanding of the chemical and physicochemical properties of the proteins and their interactions with toxic and chromogenic components may lead to better methods of processing and detoxification. Mustard proteins consist of two protein fractions: a high molecular weight protein fraction (12S fraction) constituting about 25% and a low molecular weight protein fraction constituting about 70% of the total proteins (Gururaj Rao et al., 1978). The low molecular weight protein fraction consists of three to four proteins that are very similar in size but differ in their electrophoretic mobility.

There are reports in the literature regarding the isolation and characterization of low molecular weight protein from *Brassica napus* L., the rapeseed (Bhatty et al., 1968; Swanljung, 1972; Lonnerdal and Janson, 1972; Mackenzie and Blakely, 1972; Schwenke et al., 1973; Simard and Boulet, 1978; Raab and Schwenke, 1984). Crouch et al. (1983) have reported the amino acid sequence of this protein and the DNA sequence of the gene. The various methods that have been reported for the isolation of 2S protein from rapeseed involve the removal of 12S protein by ammonium sulfate precipitation or gel filtration on Sephadex gels and fractionation of the low molecular weight protein by repeated exclusion chromatography on Sephadex gels followed by resolving the proteins that are differing in charge though similar in size with ion-exchange chromatography. Raab and Schwenke (1984) have isolated a low molecular weight protein fraction by means of fractional precipitation and dissolution with ammonium

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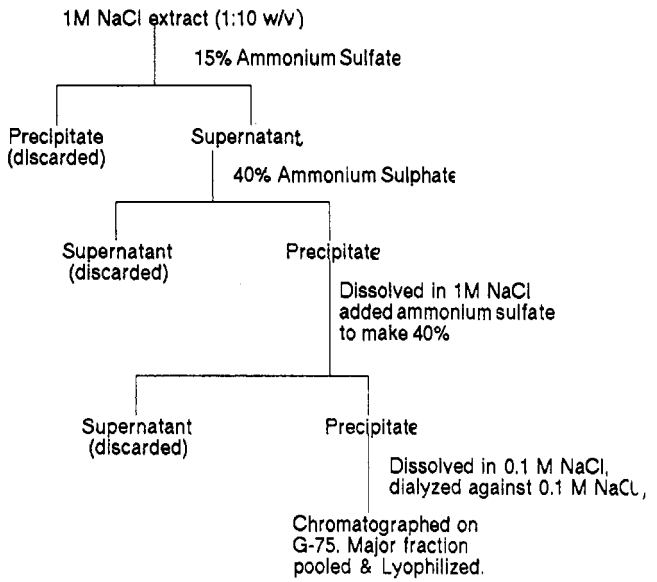

Figure 1. Schematic diagram for isolation of low molecular weight protein.

sulfate and single chromatography on Sephadex G-200. Gururaj Rao et al. (1978) have reported a method for the isolation of a low molecular weight fraction from mustard seed. The method involves the removal of 12S protein by ammonium sulfate precipitation followed by isoelectric precipitation. The 2S protein preparation thus obtained is further resolved by ion-exchange chromatography.

In this paper we report a simple method based on ammonium sulfate fractionation and gel filtration on Sephadex G-75 for the isolation of low molecular weight protein from mustard (*B. juncea*). The method gives high yields of protein purified to homogeneity. We also report some of the physicochemical properties of the low molecular weight protein.

MATERIALS AND METHODS

Mustard seeds of the variety RLM-198 (*B. juncea*) were purchased from Haryana Agriculture University, Hissar, India. The dehulled seeds were defatted by repeated extraction with hexane, ground to pass through a 60-mesh (BSS) sieve, and used. The various chemicals used were from the following sources: Sephadex G-75 (superfine, 20–50 μm) from Pharmacia Fine Chemicals; trinitrobenzenesulfonic acid (TNBS), sodium dodecyl sulfate (SDS), bovine serum albumin, egg albumin, trypsin, chymotrypsin, β-lactoglobulin, carbonic anhydrase, trypsinogen, and lysozyme from Sigma Chemical Co., St. Louis, MO; Coomassie brilliant blue from Schwarz-Mann; acrylamide from E. Merck, Munich, West Germany; bisacrylamide from Koch-Light Laboratories Ltd., Colnbrook, England; *N,N,N′,N′*-tetramethylethylenediamine (TEMED) and β-mercaptoethanol from Fluka. All other chemicals used were reagent grade.

Isolation of the 2S Protein. The schematic diagram for the isolation is shown in Figure 1. In a typical experiment, 5 g (or 7.5 g) of defatted mustard flour was stirred with 50 mL (or 75 mL) of 1 M NaCl solution for 30 min. The insoluble residue was separated by centrifugation at 3000*g* for 30 min, to the clear supernatant was added solid ammonium sulfate to 15%, and the resultant mixture was stirred for 1 h and centrifuged at 3000*g* for 30 min. Solid ammonium sulfate was added to the supernatant to raise the concentration to 40%, and the resultant mixture was stirred for 1 h. This was centrifuged at 3000*g* for 30 min. The residue (precipitate) was redissolved in 1 M NaCl and reprecipitated by addition of

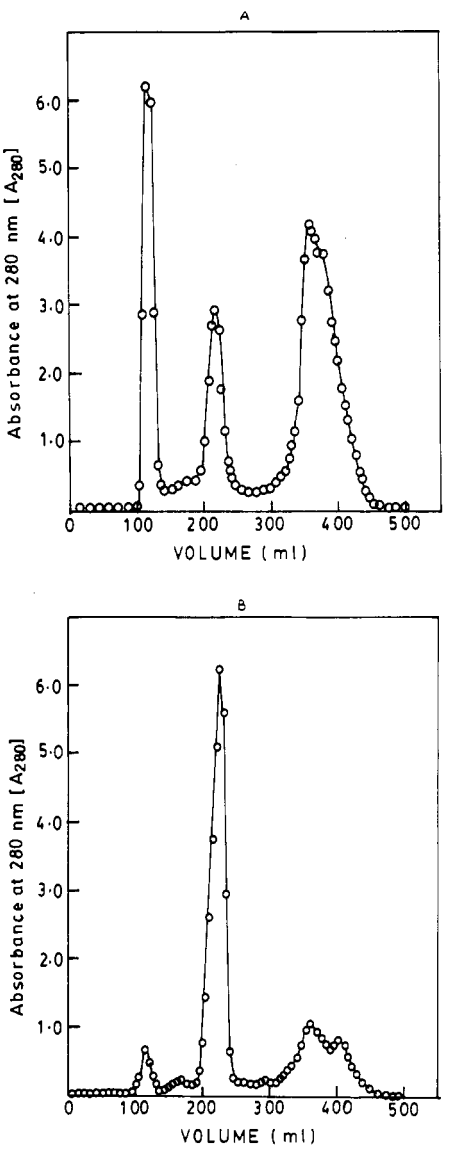

Figure 2. Gel filtration patterns: (A) unfractionated mustard proteins; (B) crude 2S protein.

40% ammonium sulfate. It was dissolved in 0.1 M NaCl and dialyzed against the same solvent to obtain this low molecular weight fraction (crude mustard 2S). The dialysate was chromatographed on a Sephadex G-75 column (45 × 3.2 cm) equilibrated with 0.1 M NaCl. Protein fractions corresponding to peak II (Figure 2B) were collected, pooled, and used for all experiments.

PAGE (Polyacrylamide Gel Electrophoresis). It was carried out according to the method of Panyim and Chalkley (1969), using 15% gels with an acrylamide to bisacrylamide ratio of 75:1 and containing 6.25 M urea at pH 3.2. Gels were stained with 0.1% amido black in 20% ethanol, 7% acetic acid, and water. They were destained in 7.5% acetic acid solution.

Determination of Subunits and Their Molecular Weights. *SDS-PAGE.* This was done according to the method of Laemmli (1970) using slab gels. Marker proteins were run along with the 2S protein, their relative mobilities calculated, and the molecular weights of the subunits obtained by plotting log *M* versus relative mobility.

Sedimentation Velocity. Sedimentation velocity measurements were made at 59780 rpm at 25 °C in a Spinco Model E analytical ultracentrifuge equipped with phase plate Schlieren optics and rotor temperature indicator and

control (RTIC) unit. A 12-mm single-sector duraluminum cell was used with a 1% protein solution in 0.1 M NaCl. $S_{20,w}$ was calculated by the standard procedure (Schachman, 1959).

Gel Filtration for Molecular Weight Determination. The molecular weight of the protein was estimated by gel filtration on a Sephadex G-75 column by the method of Andrews (1965), using the marker proteins. Sephadex G-75 was equilibrated with 0.1 M NaCl solution and was packed into the column, 1.8 × 100 cm. Elution of the protein was done with the same solvent. Fractions (3 mL) were collected, and the protein concentration was monitored by measuring the absorbance at 280 nm. The marker proteins used were bovine serum albumin (68 000), egg albumin (45 000), DFP-chymotrypsin (25 200), β-lactoglobulin (36 800), and lysozyme (14 500). The void volume (V_0) of the column was determined by eluting blue dextran from the column.

Protein Concentration. Protein concentration was estimated by measuring the absorbance of the solution at 280 nm. For 2S protein, a value of 4.8 for $E^{1\%}_{1\,cm}$ at 280 nm was used. This value was arrived at by measuring the absorbance of a series of solutions whose concentrations were determined by the Kjeldahl nitrogen to protein.

Absorption Spectrum. The ultraviolet (UV) absorption spectrum of the protein was recorded at room temperature (~30 °C) in a Beckman DU-8B spectrophotometer in the range of 220–370 nm.

Fluorescence Spectrum. The fluorescence spectrum of the protein was measured in an Amino-Bowman spectrofluorimeter at 25 °C. The emission spectrum was measured in the range of 300–400 nm after excitation at 280 nm. Protein solutions of 0.01–0.02% were used for these measurements.

Optical Rotatory Dispersion (ORD) and Circular Dichroism (CD) Spectra. These measurements were made with a Jasco J 20C automatic recording spectropolarimeter calibrated with camphor-d_{10}-sulfonic acid at room temperature (~30 °C) for CD and a sucrose solution for ORD. Quartz cells (1 cm) were used in the near-UV region and 1-mm cells in the far-UV region. Slits were programmed to yield 10-Å bandwidth at each wavelength. Mean residue rotations ($[\alpha]_{MRW}$) and mean residue ellipticity (θ_{MRW}) (deg cm^2 dmol^{-1}) were calculated by standard procedures (Adler et al., 1973). A value of 110 for the mean residue weight (MRW) was assumed.

Carbohydrate Content. Carbohydrate was estimated by the method of Montgomery (1961) using a 1% protein solution.

Phosphorus Content. Phosphorous was estimated by the method of Taussky and Shorr (1953) using a 1% protein solution in 0.1 M NaCl.

Available Lysine. It was estimated by the method of Hall et al. (1973) using 2,4,6-trinitrobenzenesulfonic acid (TNBS).

Tyrosine and Tryptophan. They were estimated according to the method of Edelhoch (1967).

RESULTS

Isolation of 2S Protein. The schematic diagram for the isolation of 2S protein from 1 M NaCl extract of defatted mustard flour by a combination of ammonium sulfate fractionation and gel filtration on Sephadex G-75 is shown in Figure 1. The protein that is precipitated between 15 and 40% ammonium sulfate is mostly 2S protein. At this stage of preparation the protein is not free from chromogenic impurities. The gel filtration patterns of mustard-unfractionated protein and crude 2S protein on a preparative Sephadex G-75 column are shown in Figure 2. The unfractionated proteins can be resolved into three major components: Fraction 1 corresponds to high molecular weight protein, fraction 2 corresponds to 2S protein, and fraction 3 has a λ_{max} at 324 nm, suggesting that it corresponds to phenolic acids. From gel filtration patterns, it is apparent that, after ammonium sulfate fractionation, the crude 2S preparation is enriched for the 2S protein. The crude 2S protein after passing through a G-75 column, corresponding to peak II, yields a 2S preparation that is free from the associated impurities.

Homogeneity. The homogeneity of the 2S preparation was ascertained by PAGE in 6.25 M urea at pH 3.8. The material moves as a single symmetrical sharp band in the above system (Figure 3A). In alkaline anionic buffer systems, the protein does not move, indicating the basic nature of the protein.

Sedimentation Velocity. The homogeneity of the preparation was ascertained by sedimentation velocity measurements. The protein sediments as a single peak at pH 6.0 in 0.1 M NaCl (Figure 3B) with a sedimentation coefficient of 1.7 S.

Gel Filtration. The pooled fractions from the preparative gel filtration column on rechromatography eluted as a single symmetrical peak on a Sephadex G-75 column in 0.1 M NaCl.

Determination of Molecular Weight. The molecular weight of the protein was estimated by gel filtration on a Sephadex G-75 column by the method of Andrews (1965). The molecular weight of the protein was found to be 22 900 ± 2000.

Estimation of Molecular Weight by SDS-PAGE in the Presence of β-Mercaptoethanol. The SDS-PAGE pattern of the protein consists of two bands of unequal intensity. From the plot of log M versus relative mobility, the molecular weights of the two polypeptide chains were calculated. The molecular weights of the two chains are 13 000 and 12 000. Thus the mustard 2S protein consists of two polypeptide chains held together by disulfide bonds.

Chemical Composition. The protein contains only 0.2% carbohydrate and does not contain any phosphorus.

Estimation of Available Lysine Content. The 2S protein contains 6.7 ± 0.4% lysine. Compared to other oilseed proteins, and with respect to the lysine content of the high molecular weight protein fraction, this protein contains a much higher content of available lysine.

Spectral Studies. *Absorption Studies.* The absorption spectra of the protein in 0.1 M NaCl and 6 M guanidine hydrochloride are shown in Figure 4. The protein has an absorption maximum at 278 nm with a hump at 288 nm. In 6 M guanidine hydrochloride alone, the absorption maximum shifted to 280 nm. The ratio of absorbance at 280 nm to absorbance at 260 nm was 1.35. The absence of phosphorus and the above ratio for absorbance of 280/260 nm suggest the absence of nucleic acid and associated impurities in the protein. The extinction coefficient of the protein at 280 nm, $E^{1\%}_{1\,cm}$, is 4.8. The protein contains one tyrosine and one tryptophan residue for an assumed molecular weight of 23 000.

Fluorescence Spectra. The protein has an excitation maximum at 280 nm. The fluorescence emission spectrum of the protein is shown in Figure 5. It has an emission maximum around 335 nm. The protein contains one tyrosine and one tryptophan residue per molecule. The emission spectrum is typical of a protein containing tryptophan residues that are buried in the interior of the molecule (Shifrin et al., 1971).

Circular Dichroism Spectra. The near-ultraviolet and far-ultraviolet circular dichroism spectra are shown in

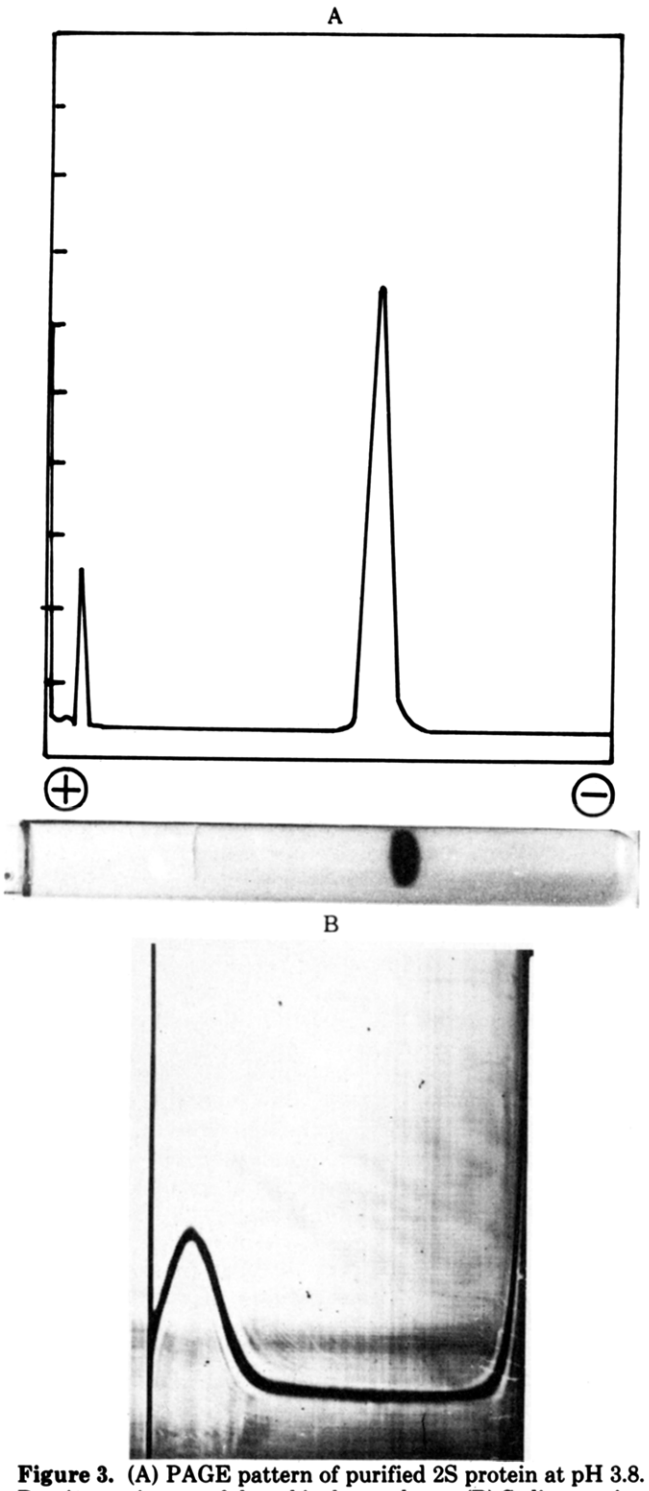

Figure 3. (A) PAGE pattern of purified 2S protein at pH 3.8. Densitometric scan of the gel is shown above. (B) Sedimentation velocity pattern of mustard 2S protein in 0.1 M NaCl.

Figure 6. In the near-UV region the protein exhibits troughs at 296, 286, and 267 nm and a peak at 290 nm. These are due to the tyrosine, tryptophan, and phenylalanine residues in the molecule. The protein contains one each of tyrosine, tryptophan, phenylalanine, and cystine residues per molecule. The overlapping contribution of the cystine residues makes the individual assignment of the bands difficult.

In the far-UV region, the protein exhibits troughs at 222 and 210 nm, typical of proteins containing α-helical sections. The α-helix content of the molecule was calculated by the method of Chen and Yang (1971) using the equation $[\theta_{222}] = -30000f_H - 2340$, where f_H is the fractional helical

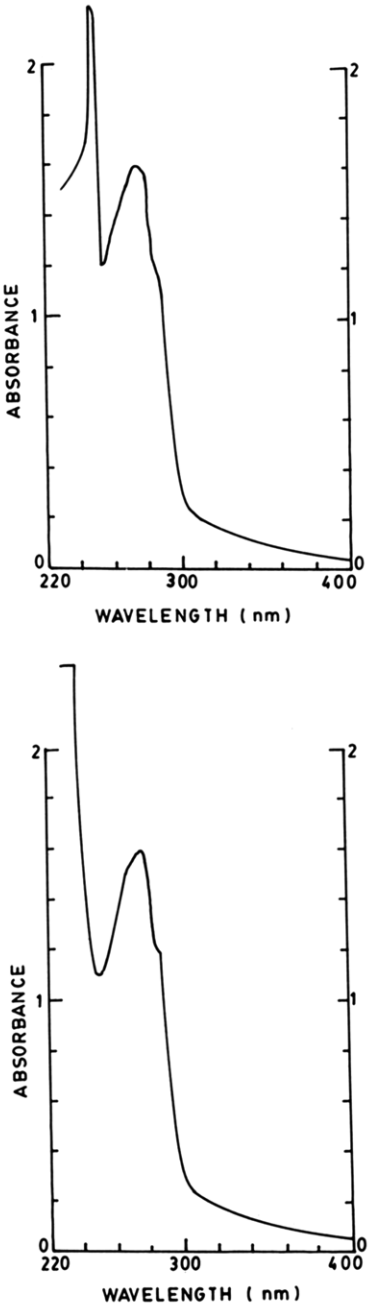

Figure 4. Absorption spectra of 2S protein in 0.1 M NaCl and 6 M Gdn·HCl in the region from 370 to 220 nm.

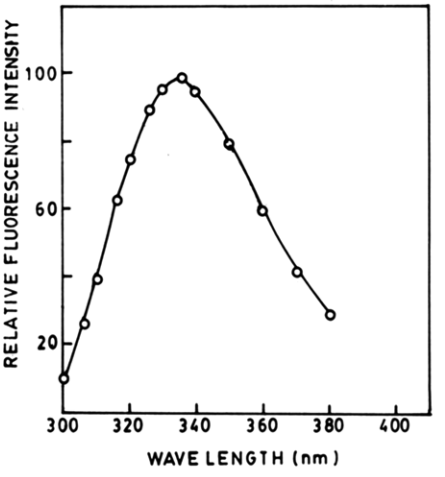

Figure 5. Fluorescence emission spectra (after activation at 280 nm).

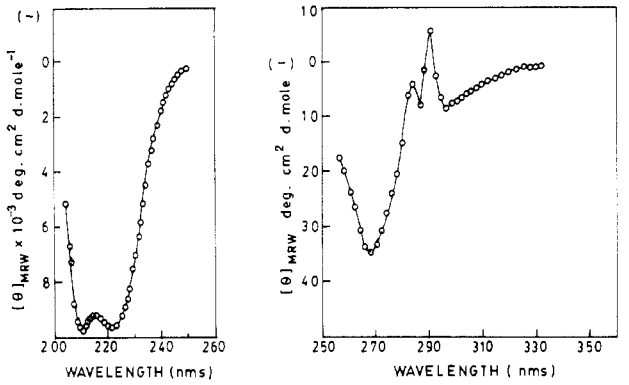

Figure 6. CD spectra of 2S protein in the near-UV and far-UV regions.

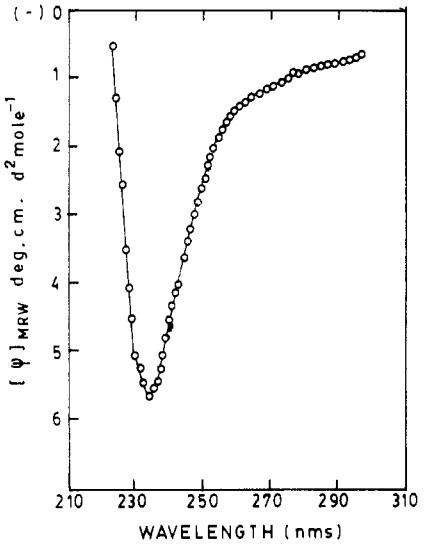

Figure 7. ORD spectra of the 2S protein in the far-UV region.

content. The protein contains 25% α-helix. The secondary structure was calculated by the method of Provencher and Glockner (1981). The protein contains 25% α-helix, 36% β-structure, and the rest aperiodic.

The ORD spectrum of the protein in the region from 290 to 210 nm is shown in Figure 7. The protein has a trough around 233 nm, with a mean residue rotation of -6000° cm^2/dmol^{-1} with a crossover point at 222 nm. The trough at 233 nm could be due to both α-helix and β-structure. From the rotation it is evident that the protein contains a very large amount of ordered structure.

Discussion. Mustard protein is made up of two major protein fractions, 11S and 2S. The 2S protein has been isolated to homogeneity by a combination of ammonium sulfate precipitation and gel filtration on Sephadex G-75. The earlier methods for the isolation of low molecular weight protein from the rapeseed, *B. napus* L., involved ammonium sulfate precipitation and size exclusion chromatography on various Sephadex gels followed by ion-exchange chromatography (Bhatty et al., 1968; Swanljung, 1972; Lonnerdal and Janson, 1972; Mackenzie and Blakeley, 1972; Schwenke et al., 1973; Simard and Boulet, 1978). The method used here is much simpler; precipitation by ammonium sulfate followed by a single-step chromatography on Sephadex G-75 resulted in a high yield of the 2S protein, which is homogeneous by the criteria of PAGE in 6.25 M urea, analytical ultracentrifugation, and gel filtration. Gel filtration on Sephadex G-75 helps to remove the associated chromogenic impurities. Further, during the course of the isolation of 2S protein, the associated chromogenic impurities like phenolic acids do not interact covalently with the protein. It was essential to obtain a 2S preparation free from associated chromogenic impurities for further studies on protein–phenolic interactions that are in progress in this laboratory.

The 2S protein isolated by this method is a major component of mustard, *B. juncea*. It has a higher molecular weight (22 900 ± 2000) than the reported molecular weight (14 000–17 000) of the 2S protein from rapeseed, *B. napus* L. (Mieth et al., 1983). The SDS-PAGE pattern of two polypeptides in the presence of β-mercaptoethanol suggests the presence of two polypeptide chains held together by S–S linkage in the molecule. Similar observations have been made in the case of low molecular weight protein from rapeseed as well (Lonnerdal and Janson, 1972).

The protein contains very little carbohydrate (0.2%) and is free from phosphorus. The 12S protein also contains little carbohydrate (Schwenke et al., 1981; Gururaj Rao et al., 1978). Gururaj Rao et al. have previously reported a higher content of carbohydrate (1.8%) in their preparation of 2S fraction. Comparison of the results becomes difficult in view of the reported heterogeneity of the sample. The 2S protein contains 7.2% lysine (unpublished results), and the available lysine content is 6.7 ± 0.4%. Mackenzie and Blakely (1972) have reported a lysine content of 3.8% for the 12S protein. The 2S protein from rapeseed, *B. napus* L., contains 8.3% lysine (Mieth et al., 1983). Because of the already high proportion of the 2S protein in mustard and rapeseed, and the higher content of lysine in these proteins, the 2S protein could be a good prospect for genetic manipulation. In other oilseeds as well, where the 2S protein forms a much less proportion (20%) of the total protein, the low molecular weight protein has a much higher lysine content than the high molecular weight protein (Prakash and Narasinga Rao, 1986).

The spectral measurements indicate the presence of one tyrosine and one tryptophan residue per molecule. This is in agreement with the reported value of tyrosine and tryptophan content of the low molecular weight protein fraction from *B. juncea* (Mackenzie and Blakeley, 1972) and the low molecular weight protein from rapeseed (Mieth et al., 1983). Even with one tryptophan residue, the fluorescence emission maximum is typical of a protein containing tryptophan in the interior of the molecule. The shoulder observed at 288 nm in the absorption spectra suggests the predominance of tryptophan contribution for the UV spectrum of the protein. The presence of one tyrosine and one tryptophan residue offers a convenient tool for following protein–ligand interactions by different spectroscopic methods.

The near-UV CD spectra of the 2S protein differ considerably from that of the 12S protein of *B. juncea* (Kishore Kumar Murthy et al., 1986), suggesting a difference in their tertiary structure. The far-UV CD data of the 2S protein indicate the presence of more extensive ordered structure in the molecule (the sum of the α-helix and β-structure is 62%), which is in contrast to the far-UV CD spectrum of the 12S protein from *B. juncea* (Gururaj Rao and Narasinga Rao, 1981; Kishore Kumar Murthy et al., 1986). The far-UV CD spectrum of the protein is comparable to the CD spectrum of other low molecular weight protein fractions from rapeseed (Schwenke et al., 1973) and other oilseeds (Srinivas and Narasinga Rao, 1987; Madhusudhan and Narenda Singh, 1983). The ORD spectrum of the protein also suggests a higher content of ordered structure in the molecule.

ABBREVIATIONS USED

Optical rotatory dispersion, ORD; circular dichroism, CD; β-mercaptoethanol, β-RSH; sodium dodecyl sulfate,

SDS; polyacrylamide gel electrophoresis, PAGE.

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LITERATURE CITED

Adler, A. J.; Greenfield, N. J.; Fasman, G. D. "Circular Dichroism and Optical Rotatory Dispersion of Proteins and Polypeptides". *Methods Enzymol.* **1973**, *27*, 675–767.

Andrews, P. "The Gel Filtration Behavior of Proteins Related to Their Molecular Weight". *Biochem. J.* **1965**, *96*, 595–606.

Bhatty, R. S.; Mackenzie, S. L.; Finlayson, A. J. "The Proteins of Rapeseed (*Brassica napus* L.) Soluble in Salt Solutions". *Can. J. Biochem.* **1968**, *46*, 1191–1197.

Chen, Y. H.; Yang, J. T. "A New Approach to the Calculation of Secondary Structures of Globular Proteins by Optical Rotatory Dispersion and Circular Dichroism". *Biochem. Biophys. Res. Commun.* **1971**, *44*, 1285–1291.

Crouch, M. L.; Tenbarge, K. M.; Simon, A. E.; Ferl, R. "cDNA Clones for *Brassica napus* Seed Storage Proteins: Evidence from Nucleotide Sequence Analysis That Both Subunits of Napin Are Cleaved from a Precursor Polypeptide". *J. Mol. Appl. Genet.* **1983**, *2*, 273–283.

Edelhoch, H. "Spectroscopic Determination of Tryptophan and Tyrosine in Proteins". *Biochemistry* **1967**, *6*, 1948–1954.

Goding, L. A.; Bhatty, R. S.; Finlayson, A. J. "The Characterization of the 12S 'Globulin' from Rapeseed and Its Glycoprotein Content". *Can. J. Biochem.* **1970**, *48*, 1096–1103.

Greenfield, N. J.; Fasman, G. D. "Computed Circular Dichroism Spectra for the Evaluation of Protein Conformation". *Biochemistry* **1969**, *8*, 4108–4116.

Gururaj Rao, A.; Narasinga Rao, M. S. "Comparative Study of the High Molecular Weight Protein Fraction of the Mustard (*B. juncea*) and Rapeseed (*B. campestris*)". *Int. J. Pept. Protein Res.* **1981**, *18*, 154–161.

Gururaj Rao, A.; Kantharaj Urs, M.; Narasinga Rao, M. S. "Studies on the Proteins of Mustard Seed (*B. juncea*)". *Can. Inst. Food Sci. Technol. J.* **1978**, *11*, 155–161.

Hall, R. J.; Trinder, N.; Givens, D. I. "Use of 2,4,6-Trinitrobenzenesulfonic Acid for the Determination of Available Lysine in Animal Protein Concentrates". *Analyst* **1973**, *98*, 673–686.

Kishore Kumar Murthy, N. V.; Appu Rao, A. G.; Narasinga Rao, M. S. "A Spectroscopic Study of the Interaction of Allylisothiocyanate with Mustard 12S Protein". *Int. J. Pept. Protein Res.* **1986**, *28*, 462–467.

Laemmli, U. K. "Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4". *Nature (London)* **1970**, *227*, 680–685.

Lonnerdal, B.; Janson, J. C. "Studies on *Brassica* Seed Proteins. I. The Low Molecular Weight Proteins in Rapeseed. Isolation and Characterization". *Biochem. Biophys. Acta* **1972**, *278*, 175–183.

Mackenzie, S. L.; Blakeley, J. A. "Purification and Characterization of Small Molecular Weight Proteins from *Brassica juncea*, *B. nigra* and *B. hirta*". *Can. J. Bot.* **1972**, *50*, 1825–1834.

Madhusudhan, K. T.; Narendra Singh. "Isolation and Characterization of a Small Molecular Weight Protein of Linseed Meal". *Phytochemistry* **1985**, *24*, 2507–2509.

Mieth, G.; Schwenke, K. D.; Raab, B.; Brueckner, J. "Rapeseed: Constituents and Protein Products, Part I. Composition and Properties of Proteins and Glucosinolates". *Nahrung* **1983**, *27*, 675–697.

Montgomery, R. "Further Studies of the Phenol–Sulfuric Acid Reagent for Carbohydrates". *Biochem. Biophys. Acta* **1961**, *48*, 591–593.

Ohlson, R. "Rapeseed". In *New Protein Foods*; Altschul, A. M., Wilke, H. L., Eds.; Academic: New York, 1985; Vol. 5.

Panyim, S.; Chalkley, R. "High Resolution Acrylamide Gel Electrophoresis of Histones". *Arch. Biochem. Biophys.* **1969**, *130*, 337–346.

Prakash, V.; Narasinga Rao, M. S. "Physicochemical Properties of Oilseed Proteins". *CRC Crit. Rev. Biochem.* **1986**, *20*, 265–363.

Provencher, S. W.; Glockner, J. "Estimation of Globular Protein Secondary Structure from Circular Dichroism". *Biochemistry* **1981**, *20*, 33–37.

Raab, B.; Schwenke, K. D. "Simplified Isolation Procedure for the 12S Globulin and the Albumin Fraction from Rapeseed (*Brassica napus* L.)". *Nahrung* **1984**, *28*, 863–866.

Schachman, H. K. "Sedimentation Velocity". In *Ultracentrifugation in Biochemistry*; Academic: New York, 1959.

Schwenke, K. D.; Raab, B.; Uhlig, J.; Tkocz, H.; Behlke, J.; Boettger, M.; Freimuth, U. "Seed Proteins III. Isolation and Characterization of Albumins from Sunflower Seed and Rapeseed". *Nahrung* **1973**, *17*, 791–89.

Schwenke, K. D.; Raab, B.; Linow, K. H.; Paetz, W.; Uhlig, J. "Seed Proteins, Part 13. Isolation of the 12S Globulin from Rapeseed (*Brassica napus* L.) and Characterization as a 'Neutral' Protein". *Nahrung* **1981**, *25*, 271–280.

Shifrin, S.; Luborsky, S. W.; Grochowski, B. J. "L-Asparaginase from *Escherichia coli* B. Physicochemical Studies of the Dissociation Process". *J. Biol. Chem.* **1971**, *246*, 7708–7714.

Simard, C.; Boulet, M. "Fractionation of Proteins from Soybean, Faba Bean, Rapeseed and Alfalfa Leaf Using 'Fractionated' Dissolution". *Can. Int. Food Sci. Technol. J.* **1978**, *11*, 16–27.

Srinivas, H.; Narasinga Rao, M. S. "Studies on the Low Molecular Weight Proteins of Poppy Seed (*Papaver somniferum* L.)". *J. Agric. Food Chem.* **1987**, *35*, 12–14.

Swanljung, P. "Purification and Some Properties of a Group of Small Basic Proteins from Rapeseed". *Acta Chem. Scand.* **1972**, *26*, 405–407.

Taussky, H. H.; Shorr, E. "A Microcolorimetric Method for the Determination of Inorganic Phosphorous". *J. Biol. Chem.* **1953**, *202*, 675–685.

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